

I Claim:

1. A method for treating a neoplastic disorder in a patient comprising the steps of:
 - a) obtaining a sample of neoplastic tissue from the patient;
 - b) introducing the sample to a cell population capable of producing antibodies;
 - c) fixing and permeabilizing the cells;
 - d) reverse transcribing V_H and V_L mRNA of the fixed and permeabilized cells into V_H and V_L cDNA sequences;
 - e) PCR amplifying and linking the V_H and V_L cDNA sequences in a head-to-head transcriptional orientation;
 - f) PCR amplifying the linked sequences to create a population of DNA fragments which encode V_H and V_L antibody fragments;
 - g) cloning the population of DNA fragments into expression vectors and amplifying the cloned expression vectors;
 - h) selecting a subpopulation of expression vectors which encodes recombinant anti-neoplastic antibodies or antibody fragments and amplifying the subpopulation selected; and
 - i) administering the recombinant anti-neoplastic antibodies or antibody fragments to the patient.
2. The method of claim 1 wherein the neoplastic disorder is selected from the group consisting of leukemias, lymphomas, sarcomas, carcinomas, neural cell tumors, squamous cell carcinomas, germ cell tumors, metastases, undifferentiated tumors, seminomas, melanomas, neuroblastomas, mixed cell tumors, neoplasias caused by infectious agents and other malignancies.
3. The method of claim 1 wherein the patient is a human.
4. The method of claim 1 wherein the neoplastic tissue is selected from the group consisting of tumor derived tissues, blood and blood derived tissues, biopsied tissues, cancerous tissues, malignant tissues, metastasized tissues and combinations thereof.

5. The method of claim 1 wherein the neoplastic tissue is first propagated in an immunodeficient mouse which is selected from the group consisting of nude mice, SCID mice, chemically treated mice and sub-lethally irradiated mice.

5 6. The method of claim 1 wherein the cell population is selected from the group consisting of populations of murine cells, ovine cells, porcine cells, primate cells, human cells, transformed cells, fused cells and combinations thereof.

7. The method of claim 1 wherein the cell population is fixed with a composition containing a chemical selected from the group consisting of formaldehyde, carbohydrazide, glutaraldehyde, osmium tetroxide and combinations thereof.

10 8. The method of claim 1 wherein the cell population is permeabilized with a composition containing a chemical selected from the group consisting of Nonidet P-40, Brij, Tween, polysorbate, Triton X-100, CHAPS, sorbitan and combinations thereof.

15 9. The method of claim 1 wherein the V_H and V_L mRNA are reverse transcribed, PCR amplified and linked within each cell of the population.

10 10. The method of claim 1 wherein a primer used for PCR amplification contains a sequence specific to immunoglobulin genes.

11. The method of claim 1 wherein linking is accomplished by the hybridization of a sequence on each V_H cDNA to a complementary sequence on each V_L cDNA.

20 12. The method of claim 11 wherein the V_H and the V_L cDNAs are cloned at a restriction enzyme site present in the hybridized complementary sequence.

13. The method of claim 1 wherein the expression vectors are selected from the group consisting of plasmids, phages, cosmids, phagemids, viral vectors and combinations thereof.

14. The method of claim 13 wherein the phages are display phages.

15. The method of claim 1 wherein after cloning, the expression vectors express a recombinant antibody selected from the group consisting of the murine classes IgG₁, IgG_{2A}, IgG_{2B}, IgM, IgA, IgD and IgE, the human classes IgG₁, IgG₂, IgG₃, IgG₄, IgM, IgA₁, IgA₂, IgD and IgE, and parts or combinations thereof.

16. The method of claim 1 wherein the antibody fragments are Fab fragments.

17. The method of claim 1 wherein the subpopulation of expression vectors is selected by adsorbing the expressed antibodies or antibody fragments against a non-neoplastic tissue.

18. The method of claim 1 wherein the subpopulation of expression vectors is selected by adsorbing the expressed antibodies or antibody fragments against the neoplastic tissue.

19. The method of claim 1 wherein the recombinant antibodies or antibody fragments are administered parenterally, sublingually, rectally or enterally.

20. The method of claim 19 wherein parenteral administration is by intravenous injection, subcutaneous injection, intramuscular injection, intra-arterial injection, intrathecal injection, intraperitoneal injection or direct injection into a site of the neoplasm.

21. The method of claim 1 further comprising the step of transferring the linked sequences from the expression vectors in-frame into expression vectors which encode antibody constant regions.

22. The method of claim 1 further comprising the step of administering an anti-neoplastic agent to the patient to augment the patient's own immune system.

23. The method of claim 22 wherein the anti-neoplastic agent is selected from the group consisting of T cell growth factors, B cell growth factors, granulocyte/macrophage growth

factors, granulocyte growth factor, macrophage growth factor, stem cell growth factor, transforming growth factor, erythropoietin, bone morphogenic proteins, differentiating agents, interleukins, interferons, hormones, components of the complement system and combinations thereof.

24. The method of claim 1 further comprising the step of administering a chemotherapeutic agent to the patient.

25. The method of claim 24 wherein the chemotherapeutic agent is selected from the group consisting of alkylating agents, purines and pyrimidine analogs, vinca and vinca-like alkaloids, etoposides and etoposide-like drugs, antibiotics, corticosteroids, nitrosoureas, antimetabolites, platinum based cytotoxic drugs, hormonal antagonists, anti-androgens and anti-estrogens.

26. The method of claim 1 wherein the neoplastic tissue is obtained from one patient and the antibodies or antibody fragments are administered to a different patient.

27. The method of claim 1 wherein the expression vectors which each encode the antibody constant region also encode a toxic substance linked to the encoded recombinant anti-neoplastic antibodies or antibody fragments.

28. The method of claim 27 wherein the toxic substance is selected from the group consisting of animal toxins, plant toxins, bacterial toxins, fungal toxins, viral toxins and parasitic toxins.

29. The method of claim 27 wherein the toxic substance is selected from the group consisting of *Pseudomonas* toxins, *Diphtheria* toxins, *Escherichia* toxins and ricin.

30. A method for the treatment or prevention of a neoplastic disorder in a patient comprising the steps of:

- a) creating a library of patient-specific, anti-neoplastic antibodies or antibody

fragments; and

- b) administering the library to the patient.

31. The method of claim 30 wherein the anti-neoplastic antibodies or antibody fragments are of the class IgG.

5 32. The method of claim 30 wherein the antibody fragments are Fab fragments.

33. A method for imaging a neoplastic disorder in a patient comprising the steps of:

- a) creating a library of patient-specific, anti-neoplastic antibodies or antibody fragments;
b) labeling the antibodies or antibody fragments with a detectable label;
c) administering the labeled library to the patient; and
d) imaging the labeled antibodies or antibody fragments in the patient.

34. The method of claim 33 wherein the antibodies or antibody fragments are of the class IgG.

35. The method of claim 33 wherein the antibody fragments are Fab fragments.

36. The method of claim 33 wherein the detectable label is selected from the group consisting of radioisotopes, stable isotopes, enzymes, fluorescent chemicals, luminescent chemicals, chromatic chemicals, metals and electrical charges.

37. A labeled library of antibodies created by the method of claim 33.

38. A method for creating a patient-specific library of anti-neoplastic antibodies comprising the steps of:

- a) obtaining a sample of neoplastic tissue from a patient;
b) introducing the sample to a cell population capable of producing antibodies;
c) reverse transcribing V_H and V_L mRNA of the cell population into V_H and V_L

cDNA sequences;

- d) PCR amplifying and linking the V_H and V_L cDNA sequences in a head-to-head transcriptional orientation;
- e) PCR amplifying the linked sequences to create a population of DNA fragments which encode V_H and V_L antibody fragments;
- f) cloning the population of DNA fragments into expression vectors and selecting a subpopulation of expression vectors which encodes recombinant anti-neoplastic antibody fragments;
- g) cloning the subpopulation of DNA fragments selected in-frame into expression vectors which encode antibody constant regions to produce intact antibody genes; and
- h) expressing the subpopulation of intact antibody genes to produce the library of patient-specific, anti-neoplastic antibodies.

39. The method of claim 38 wherein the patient is a human.

40. The method of claim 38 wherein the neoplastic tissue is selected from the group consisting of tumor derived tissues, blood and blood derived tissues, biopsied tissues, metastasized tissues, cancerous tissues, malignant tissues and combinations thereof.

41. The method of claim 38 wherein the cell population is selected from the group consisting of populations of murine cells, ovine cells, porcine cells, primate cells, human cells, transformed cells, fused cells and combinations thereof.

42. The method of claim 38 wherein the cell population is fixed and permeabilized.

43. The method of claim 38 wherein the V_H and V_L mRNA are reverse transcribed, PCR amplified and linked within each cell of the population.

44. The method of claim 38 wherein the antibodies or antibody fragments are selected from the group consisting of the murine classes IgG₁, IgG_{2A}, IgG_{2B}, IgM, IgA, IgD and IgE,

the human classes IgG₁, IgG₂, IgG₃, IgG₄, IgM, IgA₁, IgA₂, IgD and IgE, and combinations thereof.

45. The method of claim 38 wherein the expression vectors are selected from the group consisting of plasmids, phages, cosmids, phagemids, viral vectors and combinations thereof.

46. A library of polyclonal antibodies created by the method of claim 38.

47. A composition comprising a patient-specific library of anti-neoplastic antibodies or antibody fragments and a pharmaceutically acceptable carrier.

48. The composition of claim 47 wherein the antibodies or antibody fragments are selected from the group consisting of the murine classes IgG₁, IgG_{2A}, IgG_{2B}, IgM, IgA, IgD and IgE, the human classes IgG₁, IgG₂, IgG₃, IgG₄, IgM, IgA₁, IgA₂, IgD and IgE, and combinations thereof.

49. The composition of claim 47 wherein the pharmaceutically acceptable carrier is selected from the group consisting of water, saline, alcohol, polyethylene glycol, oil, polysaccharides, salts, glycerol, stabilizers, anti-oxidants, emulsifiers and combinations thereof.

50. A method for creating a library of polyclonal antibodies or antibody fragments comprising the steps of:

- a) obtaining a biological sample;
- b) introducing the biological sample to a cell population capable of producing antibodies;
- c) reverse transcribing V_H and V_L mRNA of the cell population into V_H and V_L cDNA sequences;
- e) PCR amplifying and linking the V_H and V_L cDNA sequences in a head-to-head transcriptional orientation;
- f) PCR amplifying the linked sequences to create a population of DNA

fragments which encode V_H and V_L antibody fragments;

- g) cloning the population of DNA fragments into expression vectors and amplifying the cloned expression vectors; and
- h) selecting a subpopulation of expression vectors which encodes antibodies or antibody fragments directed against the biological sample and amplifying the subpopulation selected to produce the library of polyclonal antibodies or antibody fragments.

51. The method of claim 50 wherein the biological sample is selected from the group consisting of tumor-derived tissues, blood and blood-derived tissues, biopsied tissues, malignant tissues, infected tissues, bacteria, viruses, fungi, parasites, genetically abnormal tissues and combinations thereof.

52. The method of claim 50 wherein the cell population is selected from the group consisting of populations of murine cells, ovine cells, porcine cells, primate cells, human cells, transformed cells, fused cells and combinations thereof.

53. The method of claim 50 wherein the cell population is fixed and permeabilized.

54. The method of claim 50 wherein the V_H and V_L mRNA are reverse transcribed, PCR amplified and linked within each cell of the population.

55. The method of claim 50 wherein the recombinant antibodies or antibody fragments are selected from the group consisting of the murine classes IgG₁, IgG_{2A}, IgG_{2B}, IgM, IgA, IgD and IgE, the human classes IgG₁, IgG₂, IgG₃, IgG₄, IgM, IgA₁, IgA₂, IgD and IgE, and combinations thereof.

56. The method of claim 50 wherein the expression vectors are selected from the group consisting of plasmids, phages, cosmids, phagemids, viral vectors and combinations thereof.

57. A library of polyclonal antibodies or antibody fragments made by the method of

claim 50.

58. The library of claim 57 wherein the antibodies or antibody fragments are of the class IgG.

59. The library of claim 57 wherein the antibody fragments are Fab fragments.

5 60. The library of claim 57 wherein the antibodies or antibody fragments are labeled with a detectable label.

61. The library of claim 60 wherein the detectable label is selected from the group consisting of radioisotopes, stable isotopes, enzymes, fluorescent compounds, luminescent compounds, chromatic compounds, metals and electrical charges.

10 62. A diagnostic kit for the detection of an disease or disorder in a patient comprising a library of antibodies or antibody fragments made by the method of claim 50.

63. The diagnostic kit of claim 62 wherein the disease to be detected is a bacterial, viral, parasitic or mycotic infection.

64. The diagnostic kit of claim 62 wherein the disease to be detected is a neoplasm.

15 65. The diagnostic kit of claim 62 wherein the disorder to be detected is a genetic defect or deficiency.

66. The diagnostic kit of claim 62 wherein the patient is a human.

67. The diagnostic kit of claim 62 wherein the antibodies or antibody fragments are of the class IgG.

20 68. The diagnostic kit of claim 62 wherein the antibody fragments are Fab fragments.

69. The diagnostic kit of claim 62 wherein a biological sample is added to the kit to detect the disease or disorder.

70. The diagnostic kit of claim 69 wherein the biological sample is a fluid selected from the group of fluids consisting of blood, urine, bile, cerebrospinal fluid, lymph fluid, amniotic fluid and peritoneal fluid.

71. The diagnostic kit of claim 62 further comprising a secondary antibody labeled with a detectable label.

72. The diagnostic kit of claim 62 further comprising a stabilizing agent selected from the group consisting of water, saline, alcohol, polyethylene glycol, oil, polysaccharides, salts, glycerol, anti-oxidants, emulsifiers and combinations thereof.

73. A method for creating a library of genetic elements which encode V_H and V_L antibody regions comprising the steps of:

- a) obtaining a sample of tissue from a patient;
- b) introducing the sample to a cell population capable of producing antibodies;
- c) reverse transcribing V_H and V_L mRNA of the cell population into V_H and V_L cDNA sequences;
- d) PCR amplifying the V_H and V_L cDNA sequences;
- e) linking the amplified sequences in a head-to-head transcriptional orientation; and
- f) cloning the linked sequences into vectors and amplifying the vectors to create the library of genetic elements.

74. The method of claim 73 wherein the vectors are selected from the group consisting of plasmids, phages, cosmids, phagemids, viral vectors and combinations thereof.

75. A library of vectors made by the method of claim 73.

76. A composition comprising a patient-specific library of anti-neoplastic antibodies or antibody fragments and a pharmaceutically acceptable carrier.

77. The composition of claim 76 wherein the antibodies or antibody fragments are selected from the group consisting of the murine classes IgG₁, IgG_{2A}, IgG_{2B}, IgM, IgA, IgD and IgE, the human classes IgG₁, IgG₂, IgG₃, IgG₄, IgM, IgA₁, IgA₂, IgD and IgE, and combinations thereof.

78. The composition of claim 76 wherein the pharmaceutically acceptable carrier is selected from the group consisting of water, saline, alcohol, polyethylene glycol, oil, polysaccharides, salts, glycerol, stabilizers, anti-oxidants, emulsifiers and combinations thereof.

79. A method for creating a library of receptor proteins comprising the steps of:

- a) obtaining a sample of biological tissue;
- b) introducing the sample to a cell population capable of producing the receptor proteins;
- c) reverse transcribing variable region mRNAs of the receptor proteins into cDNA sequences;
- d) PCR amplifying and linking the cDNA sequences in a head-to-head transcriptional orientation;
- e) PCR amplifying the linked sequences to create a population of DNA fragments which encode a variable region of the receptor proteins;
- f) cloning the population of DNA fragments into expression vectors and amplifying the cloned expression vectors; and
- g) selecting a subpopulation of expression vectors which encodes the recombinant receptor proteins and amplifying the subpopulation selected to produce the library of receptor proteins.

80. The method of claim 79 wherein the receptor proteins are selected from the group consisting of T-cell receptors, B-cell receptors, natural killer cell receptors, macrophage

receptors and parts and combinations thereof.

81. The method of claim 79 wherein the biological tissue is selected from the group consisting of normal tissue, neoplastic tissue, infected tissue and abnormal tissue.

82. The method of claim 79 wherein the cell population is fixed and permeabilized.

5 83. The method of claim 79 wherein the V_H and V_L mRNA are reverse transcribed, PCR amplified and linked within each cell of the population.

84. The method of claim 79 wherein the vectors are selected from the group consisting of plasmids, phages, cosmids, phagemids, viral vectors and combinations thereof.

10 85. The method of claim 79 further comprising the step of transferring the linked sequences from the expression vectors in-frame into expression vectors which encode T cell receptor constant regions.

86. An expression vector comprising restriction enzyme recognition sites for insertion of two gene sequences in a head-to-head transcriptional orientation.

15 87. The expression vector of claim 86 wherein the vector is selected from the group consisting of plasmids, phages, cosmids, phagemids, viral vectors and combinations thereof.

88. The expression vector of claim 86 which is selected from the group consisting of a prokaryotic vector, a eukaryotic vector, a chimeric vector, a dual vector, a surface display vector and a combination thereof.

20 89. The expression vector of claim 86 further comprising transcription and translation control sequences.

90. The expression vector of claim 89 wherein the transcription control sequence is

selected from the group consisting of a promoter, an RNA polymerase initiation site, an RNA polymerase termination site, a TATA box, a CAT box, a poly A addition site, an enhancer and a part or combination thereof.

91. The expression vector of claim 89 wherein the translation control sequence is selected from the group consisting of a ribosome binding site, a leader sequence and a part or combination thereof.

92. The expression vector of claim 86 which is selected from the group consisting of pMDV, pCDV, *pLPP2*, pUC19-C κ -CH1, pUC119-C κ -CH1 and pJS.

93. A method for transferring a library of nucleic acid fragments between different vectors without significant loss of library diversity comprising the steps of:

- a) inserting the library of fragments into first vectors in a head-to-head transcriptional orientation;
- b) obtaining the library from the first vectors; and
- c) reinserting the library of fragments obtained into second vectors without significant loss of library diversity.

94. The method of claim 93 wherein the library comprises cDNA fragments.

95. The method of claim 93 wherein the first vectors are prokaryotic expression vectors.

96. The method of claim 93 wherein the second vectors are eukaryotic expression vectors.

97. The method of claim 93 wherein the library is inserted by ligation into first vectors.

98. The method of claim 93 wherein the library is obtained by cleaving the first vectors with one or more restriction enzymes.

99. The method of claim 93 wherein the library is obtained by PCR amplification of the inserted sequences.

100. The method of claim 93 wherein the library is reinserted by ligation into second vectors.

5 101. The method of claim 93 wherein the library comprises at least 100 different fragments.

102. The method of claim 93 wherein the library comprises at least 10^8 different fragments.

103. The method of claim 93 wherein library diversity is reduced by less than 50% after the transfer.